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Chemical profiling of the street cocktail drug 'nyaope' in South Africa using GC–MS II: Stability studies of the cannabinoid, opiate and antiretroviral components during sample storage

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ABSTRACT

Nyaope is a mixture of low grade heroin, cannabis products, antiretroviral drugs and other materials added as bulking agents. It is a highly physically additive mixture which is smoked by users. As part of the development of a method for the analysis and profiling of nyaope this study evaluates the stability of the cannabinoid, opiate and antiretroviral components of nyaope during storage following seizure. Conditions used were those typically used for storage of drug seizures: in a desiccator in a refrigerator, in a desiccator in the dark at room temperature, in a desiccator in daylight at room temperature and ambient room temperature in the dark in a cabinet used for storage of drug seizures. Street samples of cannabis (Δ^9-tetrahydrocannabinol) and heroin were mixed with efavirenz and nevirapine tablets to mimic a nyaope sample. The samples were homogenized and transferred into glass bottles and extracted with tertiary butyl alcohol (tBuOH) and analysed by gas chromatography – mass spectrometry (GC–MS) after the powdered drugs had been stored for intervals of 0 and 24 h under each storage condition. The data obtained indicates that the target drug components in nyaope samples decompose and that for comparison purposes the drug extracts should be prepared in tBuOH immediately after seizure because of the decomposition of the drug components during storage prior to extraction and analysis. The implications of this work are that law enforcement agencies dealing with nyaope and wanting to compare drug samples may need to change their practice around how the drug is handled after seizure but prior to analysis.

1. Introduction

The abuse of the cocktail drug nyaope, in South Africa, has increased in recent years mainly amongst young African and Coloured males. This often results in these young males turning to theft, losing their jobs and/or dropping out of school. The nyaope users, who are mostly poor, often resort to criminal activities to sustain their drug habit which includes stealing anything valuable that they can lay their hands on [1–4].

Chemical comparisons of nyaope will assist relevant stakeholders in prosecuting those involved in the manufacture, trafficking and distribution of the drug. It will also allow determination of the drug and other content of the mixture so that public health awareness programmes can be developed. Nyaope contains heroin in combination with cannabis and other adulterants which may include phenacetin (PNT), caffeine, efavirenz (EFV), dextromethorphan (DTM) and nevirapine (NVP) [5,6]. Street samples of heroin have been shown to contain diamorphine (DAM) together with acetylcodeine (ACOD) and 6-monoacetylmorpine (6-MAM) [5–7]. The combination of heroin, cannabis and antiretroviral (ARV) drugs may be prepared by the drug dealers or mixed together by the users of the drugs themselves resulting in a mixture of powder and plant materials. The resulting drug cocktail is mixed with tobacco and smoked (Fig. 1).

Whilst the cannabis may now, with changes in the legislation in RSA, be grown at home it may also be bought illegally on the street. The heroin is sourced illegally. The ARV's can be derived from a number of different sources. The use of ARVs as bulking agents for nyaope has reportedly led to health professionals being robbed or even corrupt officials selling the ARVs [8–10]. HIV positive patients are also either robbed or sell the ARVs themselves thereby

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Fig. 1. Illustrative sample of nyaope.

defaulting on their treatment (ibid). It is little known that common psychiatric side-effects of efavirenz include, agitation, depersonalisation, hallucinations, disturbed dreams, mood disorders, depression, suicidality, antisocial behaviour, psychosis, catatonia and delirium [11,12]. Efavirenz has hallucination effect similar to lysergic acid diethylamide (LSD) [13] and this serve as motivation for its use with cannabis in the nyaope mixture.

Table 1 shows the structures of the significant cannabinoid, opiate and antiretroviral components of nyaope, and their degradation products, which have been used for comparison of samples in previous studies of the individual drugs. The presence or otherwise of some of these drugs, for example cannabinol, allow determination of whether the sample is degrading or not. The degradation of any components may lead to erroneous chemical comparisons being made [14]. Degradation of drug samples can occur at any stage in the life cycle of a drug case including the storage of the drug after seizure. Therefore storage conditions that minimize the degradation of the components of nyaope, which include opiates that hydrolyze, and cannabinoids which oxidise, are necessary for successful comparison of nyaope samples.

There have been a number of studies of individual components of nyaope under different storage conditions. Under long term storage, cannabis, in the absence of direct light, has been shown to degrade to a lesser extent when stored at 4 °C than at 22 °C [15]. The active ingredient, Δ^9-tetrahydrocannabinol (Δ^9-THC) degrades to the less potent cannabinol (CBN) [16,17], cannabidiol (CBD) degrades to menthane carboxylic acid [18] while tetrahydrocannabivarin (THCV) which is a propyl homolog of Δ^9-THC [19,20] degrades to cannabivarin (CBV) which is itself a homologue of CBN.

It is known that DAM hydrolyses to 6-MAM and then to morphine (MOR) [21,22]. Under long-term storage (five weeks) DAM was reported to undergo degradation (an average of 17.0%) when stored in a refrigerator. When stored at ambient temperature it degraded by an average of 23.3% [23].

ACOD is a synthetic impurity of illicit heroin usually present at concentration levels of 15–20% relative to DAM and ranges up to 45% [24]. ACOD undergoes hydrolysis to codeine (COD) and subsequently to MOR [25,26].

Efavirenz ((4*S*)-6-chloro-4-(cyclopropylethynyl)-4-(trifluromethyl)-1, 4-dihydro-2H-3, 1-benzoxazin-2-one) (EFV) was shown to be stable in methanolic solution for at least 7 days and to undergo degradation after 24 h of storage when exposed to UV light [27]. EFV undergoes hydrolysis to an intermediate carbamic acid ({4-Chloro-2-[(1S)-3-cyclopropyl-1-hydroxy-1-(triflouromethyl)prop-2-yn-1 yl]phenyl}carbamic acid) which subsequently forms efavirenz amino alcohol ((2S)-2-(2-amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol) and carbon dioxide [28]. The alcohol may undergo cyclization to form the quinoline derivative (6-chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline) [29].

The potential degradation products of nevirapine (NVP) are reported to be ethyl nevirapine and descyclopropyl nevirapine [30]. EFV stock solutions in methanol and NVP stock solution in dimethyl sulfoxide were reported to be stable after 24 h of storage at ambient temperatures, and 24 and 36 months of storage, respectively, at −20 °C [31]. EFV and NVP were reported to be stable after 3 months of storage at 4 °C and 20 °C while EFV and NVP were only stable up to 50 and 70 days respectively when stored at ambient temperatures [32].

NVP has been shown to be relatively stable when exposed to humidity, UV light and heat up to 2 days of storage [33] as well as in acetonitrile up to 48 h of storage [34].

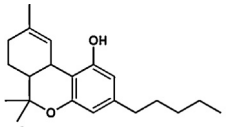
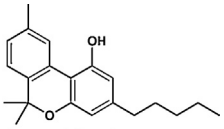
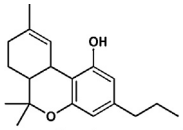
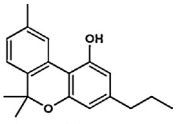
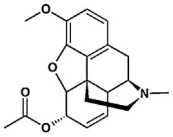
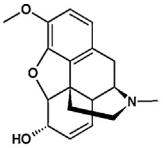
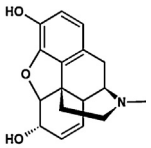
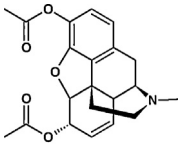
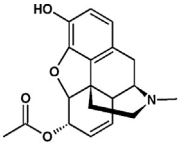
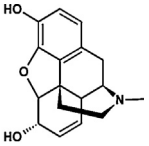
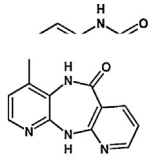
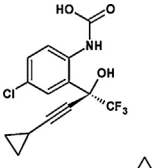
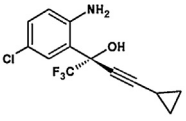
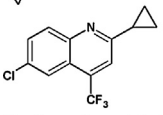
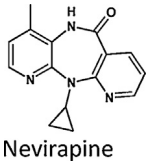
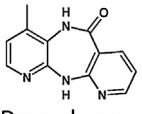
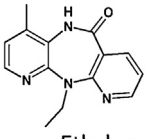
Although there is a considerable amount of literature on the long-term stability of the individual components of nyaope, to our knowledge there is no study of the stability of these compounds when mixed. This study determines the stability of the drugs found in nyaope samples over a 24 h period. This will provide information on how nyaope samples should be stored after seizure to facilitate drug identification and comparison. This in turn will inform law enforcement agencies around how such samples should be processed and how reliable data may be obtained for law enforcement purposes.

2. Material and methods

2.1. Chemicals

Tertiary butyl alcohol (t-BuOH) (ACS, Reag. Ph Eur) was purchased from Merck and tetracosane 99% was purchased from Sigma-Aldrich. Solvents were used as received without further purification. Efavirenz 600 mg tablets- PhD item 41,047 and nevirapine 200 mg tablets- PhD item 41,071 were both donated by Aspen Pharmacare. Cannabis and heroin street samples seized by the South African Police Service (SAPS) were used to formulate the simulated nyaope sample used for the study.

Table 1
Structures of the significant cannabinoid, opiate and antiretroviral components of nyaope, and their degradation products.

Component	Degradation product/s
 Δ9-Tetrahydrocannabinol Δ9-Tetrahydrocannabinol	 Cannabinol Cannabinol
 Tetrahydrocannabivarin Tetrahydrocannabivarin	 Cannabivarin Cannabivarin
 Acetylcodeine Acetylcodeine	  Codeine Morphine Codeine Morphine
 Diamorphine Diamorphine	  6-Monoacetylmorphine Morphine 6-Monoacetylmorphine Morphine
 Descyclopropyl nevi Efavirenz	   Carbamic acid derivative Efavirenz amino alcohol Quinoline derivative Carbamic acid derivative Efavirenz amino alcohol Quinoline derivative
 Nevirapine Nevirapine	  Descyclopropyl nevirapine Ethyl nevirapine Descyclopropyl nevirapine Ethyl nevirapine

2.2. Preparation of internal standards

The internal standard was prepared at a final concentration of 0.02 mg/ml in t-BuOH which was used to dissolve the samples for gas chromatography–mass spectrometry (GC–MS) analysis. Tertiary butyl alcohol has previously been shown to be the solvent of choice for the identification, comparison and profiling of nyaope samples in which, once prepared, the drugs were stable prior to analysis [7]. The internal standard solutions were subsequently used to extract the drug samples after storage at the various time intervals under the different conditions ahead of instrumental analysis.

2.3. Sample preparation

Street cannabis and heroin samples seized by the SAPS were used to prepare simulated nyaope samples. The simulated samples were prepared by mixing the heroin street sample, cannabis street sample, EFV tablet sample and NVP tablet sample, to mimic as closely as possible a typical street sample of nyaope. The heroin street samples were determined to contain caffeine, DAM, dextromethorphan (DMP), acetylcodeine (ACOD), 6-monoacetylmorphone (6-MAM), noscapine, papaverine and phenacetin (PNT) using GC–MS analysis during routine case work at the SAPS Forensic Science Laboratory (SAPS-FSL). Of these the ACOD and DAM were considered as heroin markers (since they are known to hydrolyse when they decompose) and Δ^9-THC the cannabis marker (since this is known to oxidise on decomposition) in this study.

The simulated nyaope samples were homogenised by grinding using a mortar and pestle. Each powdered nyaope sample was divided into aliquots ranging from 10.0 to 10.6 mg. To obtain data at t = 0, 10.2 mg of the homogenised samples was weighed into a 15 ml head space vial. 3 ml of the internal standard solution was added and the vial sealed. The mixture was then sonicated for 15 min [35,36]. The residue was filtered off and the eluate divided into three 800 μl portions into amber GC–MS vials, representing each of the triplicate analyses. The remainder of the aliquoted homogenised samples (Table 2) ranging between 10.0 mg–10.6 mg were weighed into clear glass bottles. The sample bottles were placed in (i) an opaque paper bag and stored in a desiccator in a refrigerator, (ii) in a desiccator at room temperature in the dark, (iii) in a desiccator under direct laboratory light (daylight) and (iv) in an uncontrolled environment in a locker (ambient). A freezer at −20 °C was not used for storage since the samples may be carried and stored for indeterminate periods prior to seizure and storage. Additionally, mobile freezers are not currently available to SAPS. The samples were subsequently extracted and analysed after storage intervals of 0, 24, 72 h and 1 and 2 weeks in the same way as the t = 0 samples.

2.4. Instrumentation

GC–MS is the analytical method of choice for drugs analysis in SAPS laboratories. Whilst LC–MS or LC–MS/MS may be theoretically preferable it is not an instrument currently available to SAPS-FSL. GC–MS analysis was carried out using an Agilent Technologies system (Chemetrix, RSA) consisting of a gas chromatograph (GC), Agilent 7890A, and mass selective (MS) detector (Agilent 5975 CVL MSD) with an auto sampler 7683 B series (1 μl injection). Chromatographic separation was performed on a computer controlled auto sampler used with a fused-silica capillary column HP-5MS (30 m × 0.25 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA, USA). Splitless injection was used at 280 °C. The GC oven temperature programme consisted of an initial temperature of 100 °C for 0.4 min, raised to 290 °C at a flow rate of 60 °C/min, held at 290 °C for 2.4 min then raised to reach 316 °C at 60 °C/min and held for 3 min. The total run time was 9.40 min. High-purity helium (99.9995%) was used as the carrier gas, at a flow rate of 1 mL/min. The MS parameters were as follows: interface temperature (280 °C), inlet temperature (250 °C), ion-source temperature (230 °C), electron ionization (EI) at 70 eV and the mass spectrometer (quadrupole) used in scan mode. The spectra were recorded in the scan range of 35–550 amu, at a scan rate of 1 scan/sec. Tetracosane (C_{24}) was used as the internal standard for GC–MS analysis. The area of the total ion chromatograph of each peak of interest was used to evaluate the stability of the samples under the respective storage condition. The samples were analysed in triplicate and the mean response, relative to tetracosane, per unit mass of drug was calculated.

Prior to analysis, confirmation that the instrument met QA standards was achieved using a system suitability test according to the SAPS-FSL protocol

3. Results and discussion

A typical separation of the components of nyaope is shown in Fig. 2 with the retention times and retention indices relative to tetracosane given in Table 3. The components DAM and Δ^9-THC were identified on the basis of their retention time and mass spectral data using certified reference material. EFV, PNT, caffeine and NVP were identified on the basis of their retention time and mass spectral data using USP reference standards while the mass spectral spectra libraries DD12 (designer drug 2012), SWGDRUG and the NIST were used to identify THCV, CBV, cannabichromene (CBC), CBD, cannabigerol (CBG), ACOD, CBN, 6-MAM, nonacosane; papaverine and noscapine. The response areas of the analytes of interest under the different storage conditions and period were determined from total ion chromatograms.

It is known that the active ingredient of cannabis, Δ^9 THC, degrades to CBN by oxidation [16]. Therefore, a measure of stability of the sample is the Δ^9-THC concentration as assessed by the relative response to the internal standard, per unit mass over time. It is also known that ACOD and DAM both hydrolyse and that the ARV's NVP and EFV also decompose. The relative responses of these compounds to the internal standard, per unit mass at t = 0 and t = 24 h are given in Table 4. All compounds listed decompose in this time frame. The greatest decomposition is seen in the ARV's with as much as 42% decomposition for NVP and 24% for EFV. With the exception of NVP, the greatest decomposition as seem under ambient conditions, as

Table 2
Mass (mg) of samples used for each time interval under the different storage conditions.

Time, hours	Samples stored in the fridge	Samples stored in the dark	Samples stored in light	Samples stored in ambient uncontrolled environment
0	10.2	10.2	10.2	10.2
24	10.4	10.3	10.3	10.2
72	10.2	10.5	10.4	10.3
168 (1)	10.6	10.3	10.1	10.2
336 (2)	10.2	10.5	10.3	10.2
672 (4)	10.2	10.3	10.3	10.6
840 (5)	10.1	10.4	10.1	10.1
1344 (8)	10.2	10.3	10.3	10.2
1512 (9)	10.1	10.1	10.5	10.5
1680 (10)	10.5	10.2	10.5	10.4
1848 (11)	10.2	10.1	10.4	10.4
2016 (12)	10.5	10.3	10.3	10.2
Average	10.28	10.29	10.31	10.29
Standard deviation	0.16	0.13	0.13	0.14
%RSD	1.65	1.27	1.27	1.46

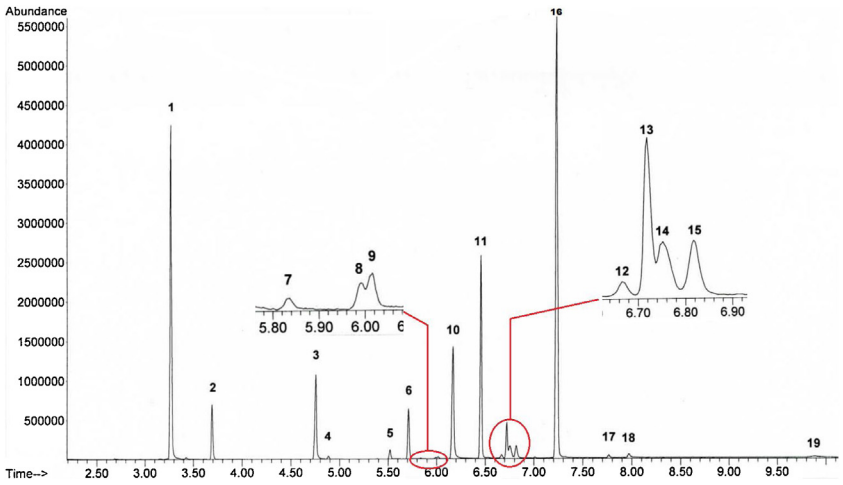


Fig. 2. Typical total ion chromatograph for the synthesised nyaope samples where (1) phenacetin; (2) caffeine; (3) efavirenz; (4) dextromethorphan; (5) tetrahydrocannabivarin; (6) tetracosane (IS); (7) cannabivarin; (8) cannabichromene; (9) cannabidiol; (10) nevirapine; (11)Δ^9-tetrahydrocannabinol; (12) cannabigerol; (13) acetylcodeine; (14) cannabinol; (15) 6-monoacetylmorphine; (16) diamorphine; (17) nonacosane; (18) papaverine and (19) noscapine.

Table 3
Retention times and relative retention indices of individual components for the simulated nyaope sample.

Component	Retention time (minutes)	Relative retention index (tetracosane = 1.000)
Phenacetin	3.266	0.572
Caffeine	3.691	0.646
Efavirenz	4.756	0.833
Dextromethorphan	4.885	0.855
Tetrahydrocannabivarin	5.520	0.967
Tetracosane	5.711	1.000
Cannabivarin	5.837	1.022
cannabichromene	5.993	1.049
Cannabidiol	6.016	1.053
Nevirapine	6.169	1.080
Δ^9-tetrahydrocannabinol	6.458	1.131
Cannabigerol	6.668	1.168
Acetylcodeine	6.723	1.177
Cannabinol	6.754	1.183
6-monoacetylmorphine	6.820	1.194
Diamorphine	7.238	1.267
Nonacosane	7.765	1.360
Papaverine	7.969	1.395
Noscapine	9.888	1.731

Table 4
Relative responses of ACOD, DMA, THC, NVP and EFV to tetracosane, per unit mass of nyaope, and percentage decomposition in 24 h.

Drug	Time (hours)	Fridge	Dark	Light	Ambient
ACOD	0	0.065	0.065	0.065	0.065
	24	0.063	0.062	0.062	0.056
	Percentage decomposition	3%	5%	5%	14%
DAM	0	0.997	0.997	0.997	0.997
	24	0.865	0.803	0.801	0.774
	Percentage decomposition	13%	20%	20%	22%
THC	0	0.352	0.352	0.352	0.352
	24	0.330	0.322	0.321	0.299
	Percentage decomposition	6.3%	8.6%	8.9%	15%
NVP	0	0.262	0.262	0.262	0.262
	24	0.151	0.190	0.156	0.176
	Percentage decomposition	42%	27%	40%	32%
EFV	0	0.185	0.185	0.185	0.185
	24	0.146	0.163	0.163	0.140
	Percentage decomposition	21%	12%	12%	24%

might theoretically be expected. Interestingly NVP decomposes most when stored in the fridge. Using the non-parametric statistic Wicoxon's Signed Ranks Test for two groups, arranged as paired observations [37], these differences are significant at the 5% level. On this basis it can be concluded that significant decomposition of the samples is occurring in as little as 24 h.

On this basis, if samples are to be analysed for comparative purposes, they should be extracted as soon as possible after seizure, in tBuOH [7]. The solution can then be safely stored prior to analysis for up to 72 h [7].

4. Conclusions

The drug markers for the profiling of cannabis include Δ^9-THC and THCV and for opiates include DAM and ACOD. This study demonstrates that when mixed together in nyaope, with the addition of the antiretrovirals, that decomposition of the drugs starts within 24 h. The implications of this are that in order to compare drug samples, extracts in tBuOH must be prepared immediately after the drug is seized, and these need to be analysed within 72 h [7]. If only drug identification is required, the drugs are qualitatively present in the samples for considerably longer time.

A further implication for drug comparison is that if two drug samples have matching drug contents then it is likely that they came from a once larger batch because it is extremely unlikely that two unrelated samples would decompose to give the same chromatogram if they were seized, extracted and analysed under identical conditions.

Conversely, if two samples have different profiles it does not mean that they were from a once larger sample or that they were not. Using the organic drug compounds it is not possible to draw a conclusion because the time of seizure and conditions of storage prior to analysis would impact on the profile. In terms of policing and forensic science this study means that, for nyaope, decisions around whether an identification is required, or a comparison is to be made, must be made at a very early stage in the case. Forensic practitioners must be aware of the requirements of the law enforcement agencies ahead of time. This may require changes in the way that nyaope cases are handled and processed by law enforcement agencies.

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